Efficiencies of recovery and extraction of trace DNA from non-porous surfaces

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Abstract

DNA recovery and extraction efficiencies are key considerations for trace DNA interpretation in casework, but prior studies have tended to focus on assessing these for body fluids rather than trace DNA. This study therefore examined the recovery and extraction of trace DNA using different collection methods from a range of non-porous surfaces relevant to crimes including homicides, terror attacks, and wildlife poaching. Direct extraction of DNA from solutions of a known concentration revealed absolute extraction efficiencies of ~82 %. When DNA was extracted from swabs seeded with the DNA solution, a similarly high efficiency of ~85 % was achieved from nylon-flocked swabs, with a lower efficiency of ~55 % from cotton swabs. However, when DNA was recovered from non-porous surfaces with swabs, ~55 % of DNA was still recovered from plastic knife handles, but lower efficiencies were achieved from the other substrates, particularly metal cable. Varied and poor recovery was observed using mini-tapes and requires further investigation. These results demonstrate that >50 % recovery efficiency of trace DNA is achievable with both swab types, although recovery rates may be affected by surface type and/or practitioner experience.

Keywords

Touch DNA; Trace DNA; Extraction efficiency; Recovery efficiency

1. Introduction

With increased sensitivity of forensic DNA profiling, trace levels of DNA left at crime scenes can now be analysed. This is particularly important for the investigation of serious crimes, such as homicides and terror attacks, and could be applied to the investigation of wildlife crime, such as illegal poaching, although this has yet to be explored. For trace DNA analysis, efficient methods are required to maximise the recovery and extraction of DNA from the surfaces examined.

Whilst a number of previous studies have focused on the effectiveness of different methods to recover body fluid DNA (e.g. [1, 2]), limited published data are available for trace DNA [3], and incorporation of recovery and extraction efficiencies are crucial steps in the interpretation of trace DNA in casework [4]. This study therefore not only investigates the efficiency of collection methods at recovering trace DNA from a range of non-porous surfaces, but also considers the efficiency of DNA extraction using QIAGEN's QIAamp® DNA Investigator Kit.

2. Materials and Methods

Background DNA was removed from substrates using 20 % bleach and UV-irradiation. Substrates represented items commonly encountered in casework: plastic-handled knives, plastic piping (e.g. used in pipe bombs), metal cable (e.g. used in poaching snares), firearm metal, and glass slides. In triplicate, aliquots of ~10 ng acellular human DNA were applied to Buffer ATL in the extraction kit to examine absolute extraction efficiency, directly to cotton and nylon-flocked swabs to examine efficiency of DNA release and extraction from swabs, and to the substrates to examine efficiency of the entire recovery and extraction process, apart from the metal cables to which ~50 ng DNA was added.

Substrates were left to dry for 24 hr before the DNA was recovered using cotton swabs (SceneSafe™), nylon-flocked swabs (COPAN's FLOQSwabs™) or mini-tapes (WA Products). A wet and dry swab protocol was employed, with 100 µl and 25 µl DNA-

free water added to cotton and nylon-flocked swabs, respectively. Substrates were sampled for 30 s: 15 s per swab or repeated applications of a single tape for 30 s.

The QIAamp® DNA Investigator Kit (QIAGEN) was used to extract DNA from the directly applied solution, swabs, and mini-tapes. DNA extracts were then quantified using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems™). The initial DNA solutions were also quantified using this kit, such that the exact quantities of DNA added (46.6 ng on the metal cables, and 9.4, 10.8 or 11.9 ng on the other samples) were used in determining recovery percentages.

3. Results

The efficiency of the QIAamp DNA Investigator kit for extracting DNA from a directly applied solution of ~10ng DNA was found to be 81.5 ± 0.7 % (Fig. 1). When the same quantity of DNA was applied to a single swab, this efficiency stayed similarly high at 84.6 ± 11.8 % from a nylon-flocked swab, but dropped to 55.8 ± 15.2 % from a cotton swab (Fig. 1). However, when known quantities of DNA were applied to a range of substrates, similar levels of recovery were seen with both swab types (Fig. 1). Approximately 55 % of the DNA applied to the plastic knife handles was recovered by both types of swabs (Fig. 1), but lower recovery efficiencies were observed from the other substrates, particularly metal cables (Fig. 1). Mini-tapes were also used to recover DNA from the glass slides, firearm metal, and plastic piping, but recovery was inefficient (<17%) and widely varied.

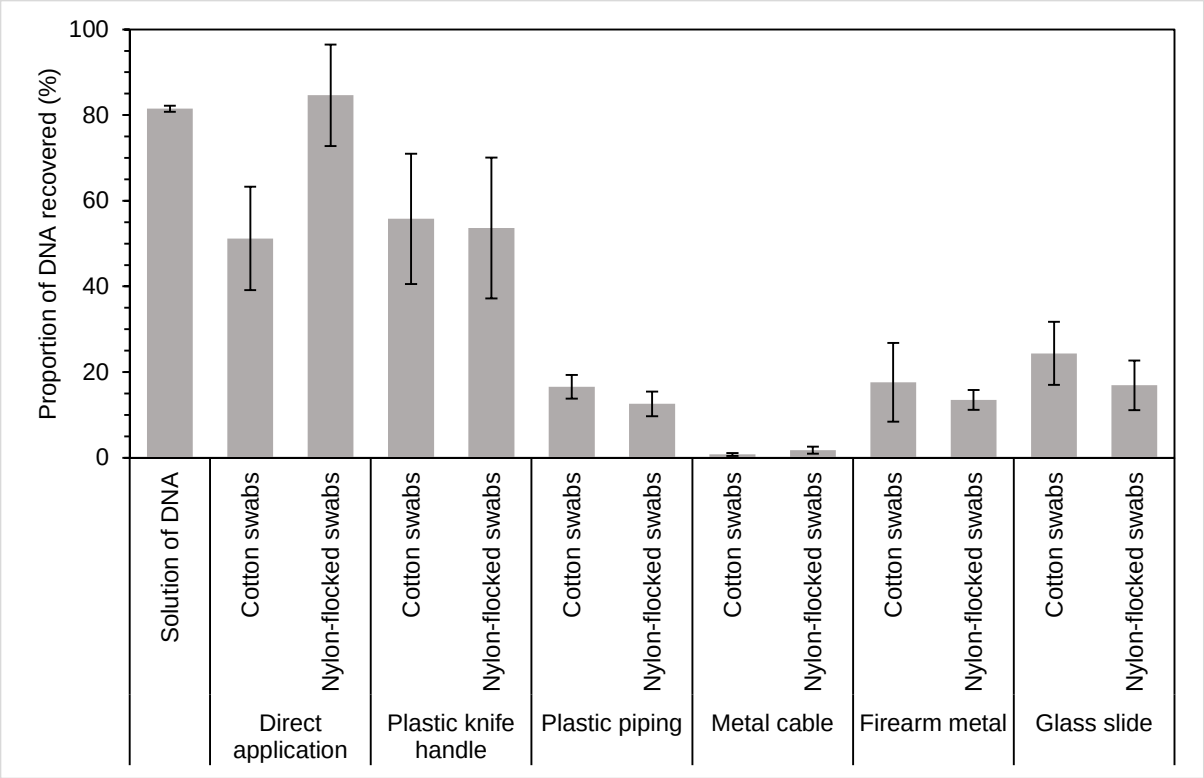

Fig. 1. Percentages of DNA recovered from a solution of known DNA quantity, from swabs that had been directly seeded with this solution, and from a range of substrates using cotton or nylon-flocked swabs.

4. Discussion

Absolute extraction efficiency, determined by extracting DNA directly from a solution of known concentration, was surprisingly high at ~80 % compared to the ~15-30 % reported in the literature (see [5] and references therein). This high efficiency was maintained when DNA was extracted from seeded nylon-flocked swabs, presumably due to their effective DNA-releasing property [6], given that a lower percentage of DNA was extracted from seeded cotton swabs. However, this difference between swab types was not seen when the swabs were used to recover DNA from a range of substrates. Using the QIAamp DNA Investigator Kit, a previous study showed that FLOQSwabs™ recovered significantly more DNA from saliva stains on petri dishes than cotton swabs [3]. The difference between that study and the results herein could

be due to differences in the DNA source used (saliva versus acellular DNA), and/or the swabbing protocol employed, since they used single wet swabs, whereas the wet and dry swab method was used here. The manufacturer of FLOQSwabs™ claims that using a single wet swab is sufficient [3], whereas it has been shown that using a wet then dry swab improves DNA recovery with cotton swabs [7].

Approximately 55 % of the DNA applied to the plastic knife handles was recovered by both types of swabs, consistent with Brownlow *et al* [3], although lower recovery efficiencies were observed with the other substrates. An experienced forensic scientist recovered the DNA from the knife handles, whereas DNA was recovered from the other substrates by newly trained individuals. This could suggest that practitioner experience may impact the efficiency of DNA recovery and is thus being investigated further. Whilst mini-tapes can successfully recover trace DNA from plastic knife handles [8], use of mini-tapes here was problematic. Significant adhesion caused occasional breakage of the glass slides and inconsistency in the number of tape applications possible during 30 s.

Although DNA can be recovered from the sheaths of metal cables left behind at scenes of metal theft [9], there are no published studies addressing the recovery of human DNA from metal cables themselves, particularly those used in wildlife crime. Here, human DNA was successfully recovered from metal cables, although the recovery was poor at <2 %. This could be due to the construction of the steel cable with a central cotton core that visibly absorbed the applied DNA solution, likely making recovery difficult. As such, DNA recovery from handled metal cables is being investigated further.

In summary, cotton swabs can be as efficient at recovering trace DNA as nylon-flocked swabs, but the rate of recovery appears to depend on practitioner experience and/or the substrate type. This, along with the variable recovery efficiency of mini-tapes, is being investigated further.

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Conflict of interest statement

None.

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